

PRE AMENDMENT
AJ
P# 6
3-11-02

PATENT

Our Docket: P-IX 4102

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:) Group Art Unit: Not yet assigned
Huse and Glaser)
Serial No.: Herewith) Examiner: Not yet assigned
Filed: Herewith)
For: ANTI- $\alpha_v\beta_3$ RECOMBINANT)
HUMAN ANTIBODIES,)
NUCLEIC ACIDS ENCODING)
SAME AND METHODS OF USE)

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Sir:

Entry of the amendments below and consideration of the
following remarks is respectfully requested.

PRELIMINARY AMENDMENT

Please amend the title page as follows:

Please delete the title starting at line 5 and insert
therefore:

COMPOSITIONS AND METHODS FOR PRODUCING ENHANCED ANTIBODIES

Please amend the specification as follows:

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On page 1, please delete the title starting at line 1,
and insert therefore:

COMPOSITIONS AND METHODS FOR PRODUCING ENHANCED ANTIBODIES

On page 14, please delete footnote 2 starting at line
11 and ending at line 12, and insert therefore:

✓² Residue numbering follows the nomenclature of Chothia et al.,
supra

On page 16, please delete the paragraph starting at
line 10 and insert therefore:

As used herein, the term "functional fragment" when
used in reference to Vitaxin, to a LM609 grafted antibody or to
heavy or light chain polypeptides thereof is intended to refer to
a portion of Vitaxin or a LM609 grafted antibody including heavy
or light chain polypeptides which still retains some or all of
the $\alpha_v\beta_3$ binding activity, $\alpha_v\beta_3$ binding specificity and/or
integrin $\alpha_v\beta_3$ -inhibitory activity. Such functional fragments can
include, for example, antibody functional fragments such as Fab,
F(ab)₂, Fv, single chain Fv (scFv). Other functional fragments
can include, for example, heavy or light chain polypeptides,
variable region polypeptides or CDR polypeptides or portions
thereof so long as such functional fragments retain binding
activity, specificity or inhibitory activity. The term is also
intended to include polypeptides encompassing, for example,
modified forms of naturally occurring amino acids such as

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On page 64, please delete the paragraph starting on line 1, and insert therefore:

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On page 83, through 84, please delete the paragraph starting at page 83, line 27, and insert therefore:

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Oligonucleotides encoding a single mutation were synthesized by introducing NN(G/T) at each CDR position as described previously (Glaser et al., *supra*). The antibody libraries were constructed in M131XL604 vector by hybridization mutagenesis as described previously, with some modifications (Rosok et al., J. Biol. Chem. 271:22611-22618 (1996); Huse et al., J. Immunol. 149:3914-3920 (1992); Kunkel, Proc. Natl. Acad. Sci. USA 82:488-492 (1985); Kunkel et al., Methods Enzymol. 154:367-382 (1987)). Briefly, the oligonucleotides were annealed at a 20:1 molar ratio to uridynylated LM609 grafted antibody template (from which the corresponding CDR had been deleted) by denaturing at 85°C for 5 min, ramping to 55°C for 1 h, holding at 55°C for 5 min, then chilling on ice. The reaction was extended by polymerization and electroporated into DH10B and titered onto a lawn of XL-1 Blue. The libraries consisted of pools of variants, each clone containing a single amino acid alteration in one of the CDR positions. Utilizing codon-based mutagenesis, every position in all of the CDRs was mutated, one at a time, resulting in the subsequent expression of all twenty amino acids at each CDR residue (Glaser et al., *supra*). The CDR libraries ranged in size from 288 (L3) to 416 (L1) unique members and contained a total of 2336 variants.

On page 87, please delete the title to the Table starting at line 8, and insert therefore:

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Table 8: Capture Lift Screening of LM609 grafted antibody CDR Libraries.

On page 87, please delete footnote 1, starting at line 18, and ending at line 20, and insert therefore:

¹Number of unique clones based on DNA sequence. Thirty-two codons are used to express all twenty amino acids at each position.

On page 97, please delete Table 10, and insert therefore:

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Table 10: Identification of Optimal Combinatorial Mutations

library*	clone	L1	L3	L3	L3	H2	H3	H3	H3	H3	k_{on} ($\times 10^4$) ($M^{-1}s^{-1}$)	k_{off} ($\times 10^{-3}$) (s^{-1})	Kd (nM)
wild type		H	G	H	L	L	Y	A	Y	Y	18.0	4.97	27.6
F32	17	F							S	S	25.1	0.138	0.5
	7	F			P	H			S	S	20.4	0.236	1.2
	56	F			P				S	S	26.6	0.135	0.5
	C59	F			P				D	D	26.5	0.137	0.5
	C176	F			P				T	T	22.5	0.192	0.9
	V357D	F							D	D	27.9	0.140	0.5
N92	C119		N		P				S	S	21.5	0.316	1.5
L96	8F9			L	P	H			S	S	47.5	0.280	0.6
	C29			L	P	H		Y	S	S	67.5	0.343	0.5
	2G4			L					S	S	60.3	0.229	0.4
	6H6			L		H			S	S	50.4	0.187	0.4
	C37			L				Y	E	E	44.8	0.147	0.3
	6D1			L	P			Y	S	S	41.0	0.158	0.4
	6G1			L	P				S	S	38.9	0.280	0.7

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Please insert new pages 101 through 130 and renumber original pages 101 through 116 as pages 131 through 146, respectively.

Please amend the claims as follows:

Cancel claim 1 without prejudice. Add new claims 105-117 as follows:

80. A grafted antibody, or functional fragment thereof, comprising an association rate constant (k_{on}) greater than $1.4 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$.

81. The grafted antibody, or functional fragment thereof, of claim 80 further comprising an association constant (K_a) greater than $5 \times 10^9 \text{ M}^{-1}$.

82. The grafted antibody, or functional fragment thereof, of claim 80, wherein said k_{on} is greater than $2.7 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$.

83. The grafted antibody, or functional fragment thereof, of claim 82 further comprising an association constant (K_a) greater than $1.0 \times 10^{10} \text{ M}^{-1}$.

84. The grafted antibody, or functional fragment thereof, of claim 80, comprising a humanized antibody, or functional fragment thereof.

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85. A method for producing an enhanced antibody, or functional fragment thereof, comprising:

(a) modifying a parent antibody, or functional fragment thereof;

(b) obtaining one or more variant antibodies, or functional fragments thereof, said one or more variant antibodies, or functional fragments thereof, comprising one or more amino acid substitutions in one or more variable regions compared to said parent antibody, and

(c) measuring the association rate constant (k_{on}) of said one or more variant antibodies, or functional fragments thereof, to an antigen, wherein a variant antibody, or functional fragment thereof, having an association rate to an antigen that is 4-fold higher or greater compared to the rate of said parent antibody binding to said antigen is an enhanced antibody, or functional fragment thereof.

86. The method of claim 85, further comprising isolating said enhanced antibody, or functional fragment thereof.

87. The method of claim 85, wherein said one or more amino acid substitutions are in one or more CDRs.

88. The method of claim 85, wherein said one or more amino acid substitutions are in one or more framework regions.

89. The method of claim 85, wherein said amino acid substitutions are in one or more CDRs and one or more framework regions.